Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*

Joan S. Steffan*, Laszlo Bodai‡, Judit Pallos†, Mamix Poelman*, Alexander McCampbell‡, Barbara L. Apostol*, Alexsey Kazantsev§, Emily Schmidt*, Ya-Zhen Zhu*, Marilee Greenwald*, Riki Kurokawa‖, David E. Housman§, George R. Jackson†, J. Lawrence Marsh† & Leslie M. Thompson*

* *Department of Psychiatry and Human Behavior, Gillespie 2121, University of California, Irvine, California 92697, USA*
† *Department of Developmental and Cell Biology, University of California, Irvine, California 92697, USA*
‡ *Neurogenetics Branch, NINDS, NIH, 10/3B14, 10 Center Drive, MSC 1250, Bethesda, Maryland 20892, USA*
§ *Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*
‖ *Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, California 92093, USA*
¶ *Department of Neurology, University of California, Los Angeles, California 90095, USA*

Proteins with expanded polyglutamine repeats cause Huntington's disease and other neurodegenerative diseases. Transcriptional dysregulation and loss of function of transcriptional co-activator proteins have been implicated in the pathogenesis of these diseases[1]. Huntington's disease is caused by expansion of a repeated sequence of the amino acid glutamine in the abnormal protein huntingtin (Htt). Here we show that the polyglutamine-containing domain of Htt, Htt exon 1 protein (Httex1p), directly binds the acetyltransferase domains of two distinct proteins: CREB-binding protein (CBP) and p300/CBP-associated factor (P/CAF). In cell-free assays, Httex1p also inhibits the acetyltransferase activity of at least three enzymes: p300, P/CAF and CBP. Expression of Httex1p in cultured cells reduces the level of the acetylated histones H3 and H4, and this reduction can be reversed by administering inhibitors of histone deacetylase (HDAC). *In vivo*, HDAC inhibitors arrest ongoing progressive neuronal degeneration induced by polyglutamine repeat expansion, and they reduce lethality in two *Drosophila* models of polyglutamine disease. These findings raise the possibility that therapy with HDAC inhibitors may slow or prevent the progressive neurodegeneration seen in Huntington's disease and other polyglutamine-repeat diseases, even after the onset of symptoms.

Huntington's disease is a late-onset neurodegenerative disease characterized by a movement disorder, neuropsychiatric symptoms and cognitive deficits caused by expansion of a glutamine repeat in the Htt protein. Currently, no cure or effective treatment for this agonizing, lethal disease exists. The aetiology of several other neurodegenerative diseases, including spinocerebellar ataxia 1 and Kennedy's disease, also involves expansion of a polyglutamine repeat[2]. The pathology of these diseases may involve transcriptional dysregulation[1]. We previously found that a fragment of mutant Htt interacts directly with CBP, which contains an acetyltransferase domain and is a co-activator of numerous promoters[3–5]. Mutant Htt represses transcription from CBP/p300 co-activated promoters in cell culture[5,6], and CBP and other transcriptional regulatory proteins are sequestered in cytoplasmic and nuclear aggregates in both transgenic mice and patient brains[5,6]. In addition, ectopic over-expression of CBP reduces polyglutamine-mediated death of cultured cells[4,7]. These observations prompted us to investigate whether Htt interacts with other acetyltransferase-containing enzymes, to

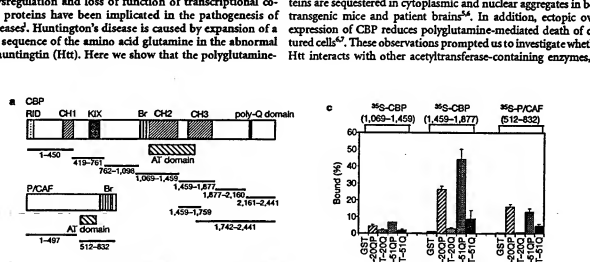

Figure 1 Htt interacts with the acetyltransferase (AT) domain of CBP and P/CAF *in vitro*. a, Schematic representations of CBP and P/CAF. RID, nuclear hormone receptor interacting domain; CH1, CH2, CH3, cysteine–histidine-rich regions 1, 2 and 3; Br, bromodomain; KIX, CREB-binding domain. The amino-acid residues used as ^{35}S-labelled probes for GST pull-down assays with GST–Htt proteins are designated below each protein diagram. b, Results of GST pull-down assays using radioactive domains of CBP and P/CAF with GST–51QP Htt. The AT domains of both CBP and P/CAF interact with GST–51QP. c, Interaction of GST–Htt fusion proteins, containing wild-type and expanded polyglutamine stretches, with and without the proline-rich domain, with radioactive probes containing the AT domains of CBP and P/CAF. A representative autoradiogram of the middle panel is shown. Slight alteration in the pattern of CBP(1,459–1,877) in the GST–51QP lane as compared with the other lanes is due to co-migration of GST–51QP with labelled CBP(1,459–1,877).

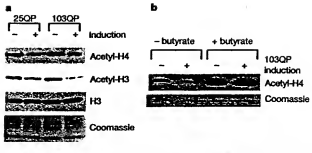

Figure 3 A reduction in acetylation of histones H3 and H4 is induced by Htt expression in cell culture. a, PC12 cells induced to express 25QP-GFP or 103QP-GFP show a reduction in the level of acetylation of both histones H3 and H4. b, A second strain of stably transfected PC12 cells, grown in the absence of butyrate but induced to express F103QP-EGFP, show a reduction in the level of acetylation of H4, which can be reversed by butyrate treatment. Equivalent levels of histones are shown by Coomassie blue staining (a and b) or with anti-histone H3 (a).

acid (SAHA) also increased acetylation of H3 and H4, reversing the decrease induced by Httex1p (data not shown). Although acetylation levels are altered, total histone levels in whole-cell extracts, determined by Coomassie blue staining of total cell lysates, are unchanged by induction of Htt proteins. Thus, expression of Httex1p causes a global reduction in acetylation of histones H3 and H4 that is reversed in the presence of HDAC inhibitors.

We found that even normal repeat Htt can bind to and inhibit acetyltransferase activity (Figs 1 and 2), so why is pathology associated with only expanded repeat Htt? Mutant Htt can be proteolytically processed; the pathogenic fragment localizes to the nucleus, where it is capable of inhibiting acetyltransferase activity[8,12,13]. However, unexpanded repeat Htt does not normally localize to the nucleus[14] and therefore is not present in the cellular compartment appropriate to inhibit nuclear CBP activity. Indeed, unexpanded polyglutamine repeats can cause pathology. For instance, unexpanded human ataxin-1 protein, which contains 30 glutamines, is normally localized to the nucleus[15]. If it is expressed at sufficiently high levels there, it can produce neurodegenerative phenotypes similar to expanded 82-glutamine ataxin-1 in either *Drosophila* or mice[15]. Thus, polyglutamine pathogenesis depends heavily on both level and location.

The reduced acetylation of histones observed in the presence of expanded repeat Httex1p *in vitro* (with or without prolines), and the subsequent reversal of this effect with HDAC inhibitors in cell culture, suggested that reduced acetyltransferase activity may be an important component of polyglutamine pathogenesis *in vivo*. Expanded polyglutamine peptides alone[16] as well as expanded repeat Httex1p (this report) are intrinsically cytotoxic and cause reduced viability and neuronal degeneration when expressed in *Drosophila* neurons. If polyglutamine pathology involves suppression of histone acetylation, then one would predict that inhibition of the deacetylation process by either of two completely independent mechanisms (for example, pharmacologically or genetically) would slow or reduce polyglutamine pathogenesis *in vivo*. To test this hypothesis, transgenic flies were engineered to express either Httex1p or just polyglutamine peptides[16] in neurons. We monitored the effects of feeding flies with the HDAC inhibitors butyrate and SAHA, and of genetically reducing the activity of their HDAC, on both lethality and degeneration of photoreceptor neurons.

Neurodegeneration is most readily observed in the fly compound eye, which is composed of a regular trapezoidal arrangement of seven visible rhabdomeres (subcellular light-gathering structures) produced by the photoreceptor neurons of each *Drosophila* ommatidium (Fig. 4j). We found that expression of Httex1p with 93 glutamines (Q93) led to a progressive loss of rhabdomeres (Fig. 4a). Rather than the normal seven visible rhabdomeres, the number of rhabdomeres seen in flies expressing Httex1p Q93 progressively declined from an average of 6.35 at day 1 to 5.13 and 4.66 at days 6 and 12 after eclosion, respectively (that is, following emergence from the pupal case as an adult). Rearing larvae that expressed Httex1p Q93 on SAHA- or butyrate-containing food reduced the level of degeneration observed (Fig. 4b, c). Expression of the Httex1p Q93 transgene results in ~70% lethality (data not shown) and early adult death (Fig. 4d). In contrast, animals fed the HDAC inhibitor SAHA show increased viability (10 μM SAHA suppresses lethality to 45%; data not shown), and early adult death is markedly repressed in a concentration-dependent manner (Fig. 4d).

The effects of HDAC inhibitors on transgenic flies expressing extended polyglutamine peptides alone (Q48) were similar to those described above: Q48 flies fed butyrate or SAHA had the same distribution of rhadomeres by day 6 as 1-day-old flies (data not shown), whereas their siblings that did not receive HDAC inhibitors showed a significant degeneration of rhabdomere number over time (average of 5.47 at day 1 versus 3.92 at day 6; Fig. 4g, j). Even when fed HDAC inhibitors only after emerging from the pupal case as adults, progressive degeneration of photoreceptor neurons was still prevented (Fig. 4h, i). Therefore, even when administered to animals already exhibiting neurodegeneration, HDAC inhibitors markedly retard (or arrest) further neuronal degeneration. Thus, HDAC inhibitors rescue pathological effects of both polyglutamine peptides and Htt exon 1 polypeptides *in vivo*.

It is possible that HDAC inhibitors might affect cellular processes other than the deacetylase pathways. As an independent test of the significance of acetylation levels in the pathogenic process, we manipulated acetylation levels genetically and examined pathology. The *Drosophila Sin3A* locus encodes a co-repressor protein that is a component of HDAC complexes[17]. We found that reducing the levels of HDAC by a partial loss of function mutant, $Sin3A^{04129}$, in heterozygotes increased the viability of Httex1p Q93 flies from 29% to 65% and led to a reduction in the extent and rate of neurodegeneration (Fig. 4e). Because this mutant allele represents a partial loss of Sin3A function, the effect on rescue of neurodegeneration may be less than that observed in the presence of HDAC inhibitors. Thus, both genetic and pharmacological reductions in the activity of HDAC reduce the rate and extent of polyglutamine-induced pathology.

To rule out the possibility that the rescue of degeneration and lethality by HDAC inhibitors was simply due to altered expression of the polyglutamine transgenes in the presence of HDAC inhibitors, we used western blotting to compare the level of transgene expression in larvae expressing exon 1 polypeptides in neurons in the presence and absence of butyrate or SAHA (Fig. 4f). Transgene expression was unaltered by the presence of HDAC inhibitors.

Although we have shown functional interactions of Htt with CBP, PCAF and p300, these results do not exclude the possibility that other acetyltransferases may be targeted, but they do suggest that

treatments that raise global levels of acetylation may be effective in ameliorating the effects of Huntington's disease and other neurodegenerative processes, even after the onset of symptoms.

The above results raise the possibility that Htt peptides can lead to reduced levels of acetylation and transcription, both by binding to acetyltransferase domains and inhibiting soluble activity, and by sequestering polyglutamine-containing transcription factors (such as CBP and others) by trapping them into aggregates. When tested *in vivo* in *Drosophila* models of polyglutamine pathogenesis, inhibition of the deacetylation process by two independent mechanisms—pharmacological (HDAC inhibitors) and genetic (reduction of Sin3A activity)—reduced degeneration of photoreceptor neurons and lethality. Other investigators have observed a reduction in acetylation induced by expanded polyglutamine-repeat proteins,

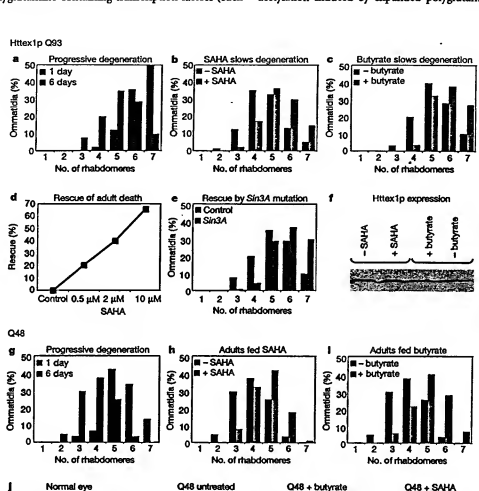

Figure 4 *In vivo* consequences of altered acetyltransferase function. **a**, The number of rhabdomeres per ommatidium at 1 and 6 d after eclosion in flies expressing Httex1p Q93 shows progressive loss. **b**, Administration of SAHA slows photoreceptor degeneration. The number of rhabdomeres per ommatidium at 6 d after eclosion is markedly improved in Httex1p-Q93-expressing flies fed SAHA. Animals were fed 0.5, 2 and 10 μM SAHA. Results with 2 μM SAHA are shown. **c**, Administration of butyrate slows neuronal degeneration. The number of rhabdomeres per ommatidium at 6 d after eclosion is markedly improved in Httex1p-Q93-expressing flies fed butyrate. Animals were fed 10, 30 and 100 mM butyrate. Results with 100 mM butyrate are shown. **d**, Administration of SAHA improves the 6-d survival of adult flies expressing Httex1p. Animals were fed 0.5, 2 and 10 μM SAHA dissolved in DMSO. Per cent rescue was calculated as follows: (per cent surviving – per cent surviving on solvent alone)/(1 – per cent surviving on solvent alone). At least 100 flies were evaluated for lethality per genotype. **e**, Genetically reducing deacetylase activity in *Sin3A* heterozygotes slows degeneration. Flies expressing Httex1p Q93 and heterozygous for a *Sin3A* mutation were compared with similar flies without the *Sin3A* mutation. The photoreceptor distribution was monitored at 6 d. **f**, Expression of the Httex1p Q93 transgene is unchanged by treatment with either SAHA or butyrate. A western blot of extracts from larvae expressing Httex1p Q93 and treated either with solvent alone or solvent with SAHA or butyrate was probed with anti-Htt antibody. Similar amounts of protein were loaded, as determined by Bradford assays and confirmed by Coomassie staining of the gel. **g**, Rhabdomeres in the eyes of flies expressing tagged Q48 peptides also show progressive loss. **h**, **i**, Progressive degeneration of photoreceptor neurons in Q48-expressing flies is arrested by 2 μM SAHA (**h**) or 100 mM butyrate (**i**) even when feeding is initiated only in adult flies. **j**, Photographs of ommatidia from Q48-expressing flies with and without HDAC inhibitors.

for example, in yeast (R. Hughes and S. Fields, personal communication) and in a cell-culture model of Kennedy's disease (A. McCampbell and K. Fischbeck, personal communication). These results strongly implicate the state of acetylation in the pathogenic process. Because several HDAC inhibitors, including SAHA[18], are currently approved by the US Food and Drug Administration (FDA) for use in other clinical settings or are in phase I clinical trials, HDAC inhibitors should be seriously considered as potential therapeutic agents for Huntington's disease and related diseases. □

Methods

Plasmid constructs

GST–Htt exon 1 fusion proteins[14]. pcDNA3.1 containing the complementary DNA for CBP[31], pGST–P/CAF-AT domain (encoding amino acids 87–765), and pcDNA3 containing the cDNA for P/CAF[28] were used. A Flag tag was cloned in front of alternating CAG/CAA repeats[11] to create F103QP-EGFP. For transgenic *Drosophila* lines, Htt exon1 constructs[16] were subcloned into pUAST[25].

GST pull-down assays

GST fusion proteins were purified and experiments were performed as described[5]. Domains of CBP were amplified by polymerase chain reaction (PCR) and the fragments cloned into pcDNA3.1. Binding and activity data were compared by an analysis of variance with StatView.

Acetyltransferase assays

The effect of Htt proteins on the acetyltransferase activity was assayed *in vitro* by a modified technique[28]. GST fusion proteins were washed in 1× HAT buffer (10 mM butyrate at pH 7.5, 10% glycerol, 50 mM Tris HCl at pH 8.0, 0.5 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.1 mM phenylmethyl sulphonyl fluoride (PMSF)), then eluted with 15 mM glutathione in 1× HAT buffer containing 50 mM NaCl. Quantified by Coomassie stain, 0.6 nmol of Htt GST fusion proteins or GST alone were incubated for 10 min at room temperature with 10 pmol of GST–CBP (amino acids 1,099–1,877), 4 pmol of GST–p300 (amino acids 1,195–1,707) (purchased from Upstate Biotechnology), or 360 pmol of GST–P/CAF (amino acids 87–832), as estimated by Bradford analysis, in a total volume of 55 μl. We then added 10 nCi ^{14}C-acetyl coenzyme A (52 mCi mmol^{-1}, NEN) and 2 μg of biotinylated N-terminal H4 (amino acids 1–21) peptide (Upstate Biotechnology) in a volume of 4 μl, and the mixture was incubated for 45 min at 30 °C. We added 500 μl of 1× HAT buffer with 30 μl of a 50% slurry of streptavidin-agarose (Upstate Biotechnology) pre-equilibrated in 1× HAT buffer. This mixture was rotated at 4 °C for 20 min, then spun in a microfuge at 2,600 *g* for 2 min. The supernatant was removed, the pellet washed twice in RIPA buffer (50 mM Tris at pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and 0.1 mM PMSF) and counted in a liquid scintillation counter. Assays were done in quadruplicate and the standard error of the mean calculated.

Histone acetylation analysis of PC12 cells

PC12 cells, stably transfected with ecdysone-inducible (Invitrogen) F103QP-EGFP (ref. 5 and our own unpublished data) were plated, induced with 5 μM ponasterone (Invitrogen) for 48 h, and treated for the last 24 h with 5 mM sodium butyrate. Controls included uninduced and/or non-butyrate-treated cells. Cells were lysed in 1× HAT buffer with 50 mM NaCl, 0.3% NP40 and 1× protease-inhibitor cocktail on ice for 10 min. Fifty micrograms of whole-cell extract were analysed by western blot. Separate, independently derived, ecdysone-inducible PC12 cell lines were also analysed. These cells, denoted PC12/pWN:Htt exQ103-EGFP cells and PC12/pWN:Htt ex1Q25-EGFP, were a gift of E. S. Schweitzer. These clonal cells contain a modified exon1 of Htt[16] inserted into an expression vector containing the *Bombyx* ecdysone-regulated element[26]. Twenty-five micrograms of whole-cell extracts from PC12 cells stably transfected with plasmids encoding inducible 25QP-EGFP and 103QP-EGFP were analysed for acetyltransferase activity. Control cells (uninduced) and cells induced with 1 μM tebufenizide for 12 h were analysed by western blot. Anti-acetylated histone H4, anti-histone H3 and anti-acetylated histone H3 (all from Upstate Biotechnology) were used to determine levels of acetylated histones H3 and H4 relative to levels of total H3.

Drosophila stocks and crosses

Expression of polyglutamine-containing peptides is driven by the bipartite expression system upstream activator sequence (UAS)–GAL4 in transgenic flies[24]. Injection of plasmids expressing Httex1p with 20 or 93 glutamines produced nine and ten lines, respectively. Two lines with the most severe neuronal phenotype (P463 and P465) were chosen for the studies reported here. Polyglutamine stocks are w; P{w^{mC} = *UAS-Q93httexon1*}^{P1} and w; P{w^{mC} = *UAS – Q48 + myc/flag*}^{P}. Constructs under the control of a yeast UAS were crossed to flies expressing the yeast GAL4 transcriptional activator[20] driven by the neuron-specific promoter *elav* (chromosome-2 driver for Q48 lines[16] and X-chromosome driver for Htt exon 1 lines[16]) that is expressed in all neurons from embryogenesis onwards: w; P{w^{mC} = +}; *elav – GAL4)C)C)O*, P{w^{mC} = *Act – GFP)JMR1C)O* actin-GFP or w; P{w$^{mW.hs}$ = *GawB)elavC155*. The inhibitor concentration ranges tested were based on cell culture experiments (SAHA, a gift from Calbiochem) or published protein effect variegation studies (butyrate)[28]. For testing the effect of *Sin3A* on polyglutamine phenotypes, w; P{w^{mC} = UAS-*Q93httexon1*}^{P1} virgins were crossed to w; P{w^{mC}=w P=w; *elav-GAL4)VI*; *Sin3A*^{06105}/*Bc Gla* males.

Pseudopupil analysis and transgene expression

Pseudopupil analysis allows visualization of the arrangement of rhabdomeres in the ommatidia of the compound eye[27]. Adult flies were decapitated, one eye was dipped in a drop of nail polish and the head was mounted on a microscope slide. Eyes were analysed with a Nikon EFD-3/Optiphot-2 scope using a × 50 objective, and photographed with a Spot camera. At least 200 ommatidia were scored for each condition. For western analysis of transgene expression, 20 larvae from each sample were ground in a buffer containing 0.1 M phosphate at pH 7.1, 0.3 M sucrose, 0.02 mM phenylthiourea, protease cocktail and 0.1 mM PMSF, and 200 μg of total lysate were loaded per lane.

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1. Cha, J. H. Transcriptional dysregulation in Huntington's disease. *Trends Neurosci.* **23,** 387–392 (2000).
2. Zoghbi, H. Y. & Orr, H. T. Glutamine repeats and neurodegeneration. *Annu. Rev. Neurosci.* **23,** 217–247 (2000).
3. Goodman, R. H. & Smolik, S. CBP/p300 in cell growth, transformation, and development. *Genes Dev.* **14,** 1553–1577 (2000).
4. Giordano, A. & Avantaggiati, M. L. p300 and CBP: partners for life and death. *J. Cell. Physiol.* **181,** 218–230 (1999).
5. Steffan, J. S. *et al.* The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl Acad. Sci. USA* **97,** 6763–6768 (2000).
6. Nucifora, F. C. *et al.* Interference by Huntingtin and Atrophin-1 with CBP-mediated transcription leading to cellular toxicity. *Science* **291,** 2423–2428 (2001).
7. McCampbell, A. *et al.* CREB-binding protein sequestration by expanded polyglutamine. *Hum. Mol. Gen.* **9,** 2197–2202 (2000).
8. Paulson, H. L. Protein fate in neurodegenerative proteinopathies: polyglutamine diseases join the (mis)fold. *Am. J. Hum. Genet.* **64,** 339–345 (1999).
9. Wanker, E. E. Protein aggregation and pathogenesis of Huntington's disease: mechanisms and correlations. *Biol. Chem.* **381,** 937–942 (2000).
10. Mangiarini, L. *et al.* Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* **87,** 493–506 (1996).
11. Kazantsev, A., Preisinger, E., Dranovsky, A., Goldgaber, D. & Housman, D. Insoluble detergent-resistant aggregate forms between pathological and nonpathological lengths of polyglutamine in mammalian cells. *Proc. Natl Acad. Sci. USA* **96,** 11404–11409 (1999).
12. Klement, I. A. *et al.* Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. *Cell* **95,** 41–53 (1998).
13. Saudou, F., Finkbeiner, S., Devys, D. & Greenberg, M. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* **95,** 55–66 (1998).
14. Sapp, E. *et al.* Huntingtin localization in brains of normal and Huntington's disease patients. *Ann. Neurol.* **42,** 604–611 (1997).
15. Fernandez-Funez, P. *et al.* Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature* **408,** 101–106 (2000).
16. Marsh, J. L. *et al.* Expanded polyglutamine peptides alone are intrinsically cytotoxic and cause neurodegeneration in *Drosophila*. *Hum. Mol. Gen.* **9,** 13–25 (2000).
17. Neufeld, T. P., Tang, A. H. & Rubin, G. M. A genetic screen to identify components of the *sina* signalling pathway in *Drosophila* eye development. *Genetics* **148,** 277–286 (1998).
18. Marks, P. A., Richon, V. M. & Rifkind, R. A. Histone deacetylase inhibitors: inducers of differentiation or apoptosis of transformed cells. *J. Natl Cancer Inst.* **92,** 1210–1216 (2000).
19. Scherzinger, E. *et al.* Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: Implications for Huntington's disease pathology. *Proc. Natl Acad. Sci. USA* **96,** 4604–4609 (1999).
20. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H. & Nakatani, Y. A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* **382,** 319–324 (1996).
21. Brand, A. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118,** 401–415 (1993).
22. Ait-Si-Ali, S., Ramirez, S., Robin, P., Trouche, D. & Harel-Bellan, A. A rapid and sensitive assay for histone acetyl-transferase activity. *Nucleic Acids Res.* **26,** 3869–3870 (1998).
23. Suhr, S. T., Gil, E. B., Senut, M. C. & Gage, F. H. High level transactivation by a modified *Bombyx* ecdysone receptor in mammalian cells without exogenous retinoid X receptor. *Proc. Natl Acad. Sci. USA* **95,** 7999–8004 (1998).
24. Luo, L., Liao, Y. J., Jan, L. Y. & Jan, Y. N. Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes Dev.* **8,** 1787–1802 (1994).
25. Lin, D. M. & Goodman, C. S. Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. *Neuron* **13,** 507–523 (1994).
26. Reuter, G., Dorn, R. & Hoffman, H. J. Bartyrate sensitive suppressor of position-effect variegation mutants of *Drosophila melanogaster*. *Mol. Gen. Genet.* **188,** 480–485 (1982).
27. Franceschini, N. in *Information Processing in the Visual Systems of Arthropods* (ed. Wehner, R.) 75–82 (Springer, Berlin, 1972).

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Correspondence and requests for materials should be addressed to L.M.T. (e-mail: lmthomps@uci.edu).